NREL-Amoco CRADA Phase 3

Bench Scale Report 2.3

Continuous Two-Stage Fermentation of Corn Fiber Hydrolyzate Supernatant by LNHST2

Project Title: Amoco-NREL **CRADA** with corn fiber
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Objective

To investigate the fermentation **performance** of the yeast LNHST2 in a two-stage continuous fermentation system **utilizing** corn fiber hydrolyzate supematant **as** a substrate. (Note: In the rest of the report, LNHST2 is referred to **as** ST2 and L1400(pLNH33) **as** LNH33.)

Materials and Methods

Yeast strain

The organism used in these studies was the genetically engineered Purdue recombinant yeast **ST2.** The seed was prepared by growing the yeast on a Petri plate with **YEPD** medium for twenty-four hours. The organism originated at Purdue University (Dr. Nancy Ho), and was supplied to NREL by Amoco Corporation.

Inoculum Preparation

A single, isolated colony **was** removed from the above agar plate **and** inoculated into 50 mL of liquid medium composed of 2% (w/w) xylose and 1% (w/w) CSL. The first stage inoculum **was** incubated at 30°C in a rotary shaker (150 **rpm)** for 30 hours. The second stage inoculum was started with a 10% inoculum **from** the first stage and lasted for **18** hours. The medium composition was the same **as** for the first stage.

GrowthMedium

The corn fiber substrate was pretreated in the APR. The liquor from the whole corn fiber slurry was separated by centrifugation in a basket centrifuge. The growth medium was prepared by **adding** 2% (w/w) corn steep **liquor (GPC)** to 15 liters of pretreated corn fiber supernatant **and** adjusting the pH to 4.5 from **an initial** pH of 1.6 **with sodium** hydroxide pellets. The medium reservoir was autoclaved at 121° C for 90 minutes, allowed to cool, **and** connected to the first fermentor **with** constant agitation **by** a magnetic stirrer.

Inoculation

The fermenter was charged **with** 900 mL of the hydrolyzate **medium** described above, and the pH was adjusted to 5.00 with 3M NaOH. The fermenter was then **inoculated with** 100 mL of the second stage inoculum. This first stage fermenter **was** initially operated in batch mode for 24 hours **and** then switched to continuous operation. The second stage fermenter was connected in series to the first one and allowed to fill with the effluent from the first stage fermenter before being switched to continuous operation.

*Growth' **Conditions***

The continuous fermentation temperature **was** maintained at 30°C and the agitation speed at 150 rpm. The steady state volume was 1000 mL with a resident time of 24 hours in each fermenter.

*Analytical **techniques***

Glucose **and** ethanol concentrations **were** determined using a Hewlett Packard 1090 **HPLC** equipped with a 1047 IR detector, **HPX-87XH, and** a HPX-87XP column. Column temperature was 85" C. **All** samples were sterilized through 0.2μ filters.

Results and Discussion

The study lasted 610 hours, but only data **from** the last 350 hours of the run are available to **date** (due to a backlog of analytical **work** by **CAT).** Ethanol concentrations in the first stage gradually fell off **from a** high of 14.1 g/L to less than 1g/L in just over 200 hours of fermentation time. **This** decline in ethanol concentrations and the concomitant increase in residual glucose and xylose suggest that wash out conditions were reached. Based on the performance of this organism in **a** similar fermentation system at the same dilution rates on pure glucose, xylose, and CSL, it is known that it can achieve far better fermentation performance **than** the one observed in the present study. Hence, it is believed that the reason for the eventual **wash** out is probably due to toxin inhibitory effects on cell metabolism. The toxins most likely responsible for this condition would have originated in the pretreatment and sterilization steps during the preparation of the substrate and the growth medium. Some of these inhibitory products are acetic acid and Maillard reaction products (reaction between **sugars** and amino groups). Indeed, the average level of acetic acid in the fresh medium **was** quite high, 7.8 g/L (higher than the usual **4-5** g/L). The highest concentration of acetic acid measured in the first stage fermenter **was** 10.3 g/L with **an** average of **7.2 g/L.**

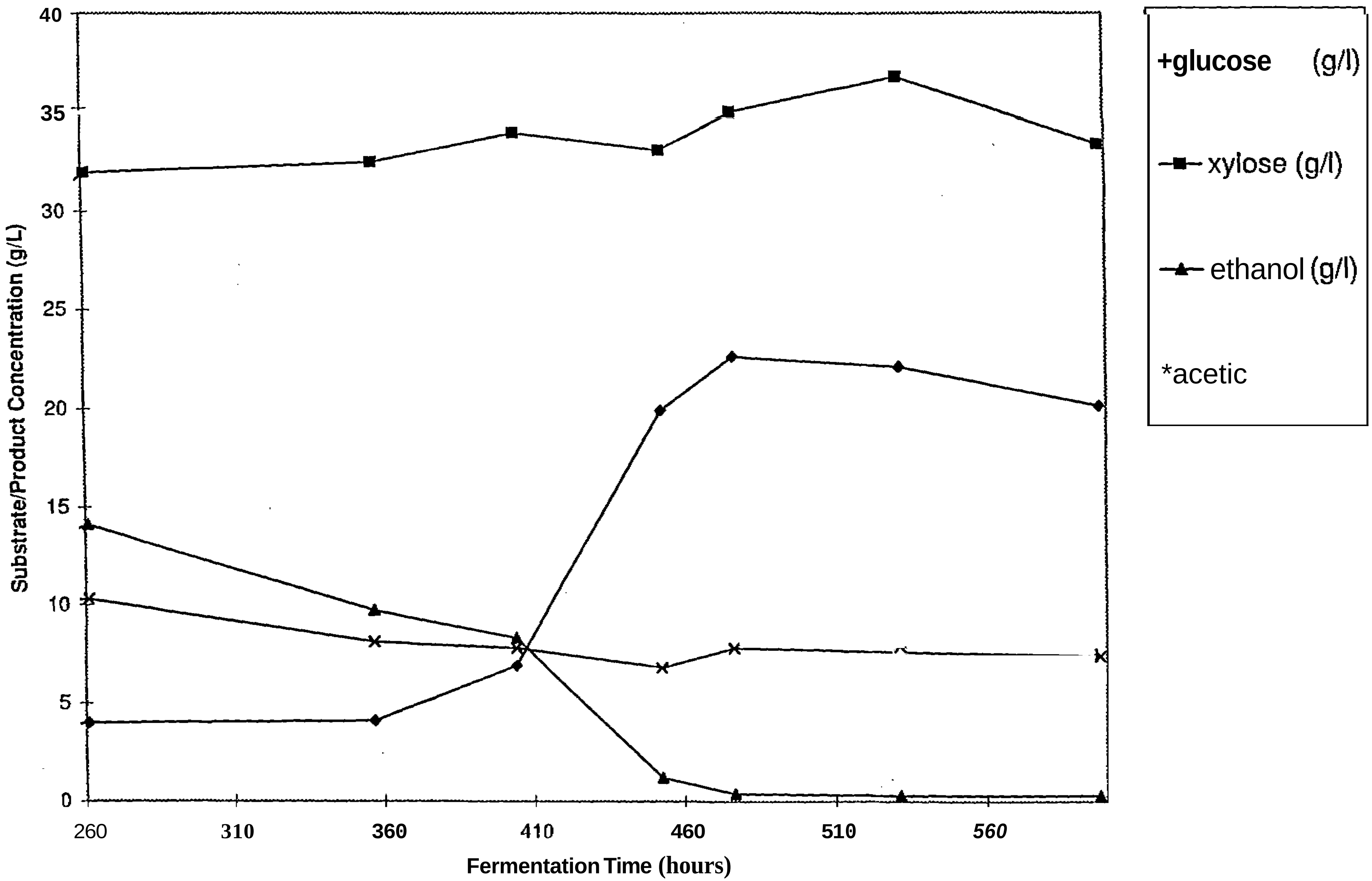
The productivity for the period between 260 and 410 hours **(Chart** 1) in the first stage fermenter was 0.5 **g/L·h** and the ethanol yield was 24% based on glucose **and** xylose consumption (process yield). The productivity for the **same period** in the second stage fermenter (Chart 2) dropped to about one-half **that** noted in the first stage and the yield **was** relatively unchanged due to the little additional fermentation taking place in the second vessel. Nevertheless, these results represent **an** improvement of 2% in yield and 138% in productivity compared to the single stage, 72-hour fermentation of hydrolyzate using LNH33. The monomeric glucose **and** xylose utilized were 81% and 13%, respectively, which demonstrates **a** capability by this yeast to cofennent glucose and xylose under harsh conditions. In contrast, LNH33 did not use any appreciable amount of xylose at an even longer residence time (72 hours).

Conclusions

The yeast ST2 outperformed the previously tested strain (LNH33) in productivity of ethanol **and** sugar utilization in the presence of inhibitory compounds (hydrolyzate). The results **from this study** show a slight increase in yield (by 2%) **and a** high **increase** in productivity (by 138%), when the hvo stage, 48-h residence time fernentation of ST2 is compared to the one-stage, **72-h** residence time fermentation of LNH33.

More importantly, ST2 can coferment glucose and xylose. Certainly, there is room for improvement, especially in light of the lack of any substantial fermentative activity in the second stage. Although the inhibitory potential of the hydrolyzate is a function of pretreatment conditions (and perhaps of sample storage conditions), the Maillard reaction products can be avoided by sterilizing the hydrolyzate separately from the CSL. Fortunately, this is not a problem at the PDU level. Undoubtedly, the promising cofermentation performance of ST2 enhances the prospects for meeting the PDU run objectives using corn fiber pretreated under optimal APR conditions, which are expected to produce no more than about 3-4 g/L of acetic acid.

Stage A Chart 1



Stage 2, Chart 2